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STRUCTURE–ACTIVITY RELATIONSHIP OF LEPIDIMOIDE AND ITS ANALOGUES

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Key Word Index—*Amaranthus caudatus* L.; Amaranthaceae; allelopathic substance; lepidimoide; structure–activity relationship.

Abstract—The structure–activity relationship of lepidimoide and its analogues was investigated by means of the *Amaranthus caudatus* L. hypocotyl elongation test. In addition, the activities of α-D-galacturonic acid and L-(+)-rhamnose, which are component sugars of lepidimoide, were also studied. The carboxylic acid free type of lepidimoide showed growth-promoting activity as high as the original lepidimoide (sodium type). The acetylated compound showed considerably higher activity than lepidimoide, whereas the methylated lepidimoide did not show any activity. The hydroxylated lepidimoide without a double bond in the C-4,5 position showed lower activity. The sugar alcohol type of lepidimoide [2-*O*-(α-D-glucopyranosyl)-L-rhamnose] showed the highest activity in all the compounds studied. α-D-Galacturonic acid, L-(+)-rhamnose and their mixtures, which are component sugars of lepidimoide, exhibited only slight or no activity, respectively. D-Glucose and the mixture of D-glucose and L-(+)-rhamnose were also slightly active or inactive. These data suggest that the active sites in the chemical structure of the lepidimoide are the uronic acid derivative bearing an α,β-unsaturated carboxylate bonded to rhamnose via an α-glucoside linkage and a double bond in the C-4,5 position in the uronic acid.

INTRODUCTION

It has recently been reported that germinated cress (*Lepidium sativum* L.) seeds secrete growth-promoting substance(s) as allelopathic factor(s) to their environment [1, 2]. A new allelopathic substance named lepidimoide was isolated from the exudates of germinated cress seeds, and its structure was identified as a uronic acid derivative, bearing an α,β-unsaturated carboxylate bonded to rhamnose via an α-glucoside linkage, by spectral data and some chemical evidence [3]. Lepidimoide was synthesized from D-glucose and α-L-rhamnose and its absolute configuration was unambiguously determined to be Na 2-*O*-L-rhamnopyranosyl-4-deoxy-α-L-threo-hex-4-enopyranosiduronate [4]. Lepidimoide promotes the shoot growth of various different species, especially of *Amaranthus caudatus* L. [3]. It also shows promoting activity on other developmental processes such as leaf development, flowering and seed production in several wild types of *Arabidopsis thaliana* [5]. Moreover, lepidimoide-like activity was also found in the exudates from seeds of various weed and crop plants [6]. Lepidimoide is widespread in the exudates from many kinds of plant species, although its amount does not differ greatly among genera or families [7]. In this paper, the structure–activity relationship of lepidimoide and its analogues was studied to reveal the chemical structure required for the growth-promoting activity.

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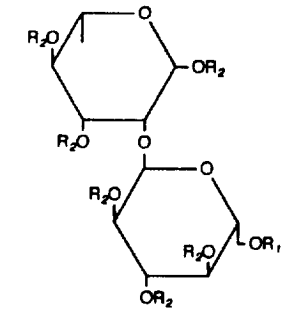
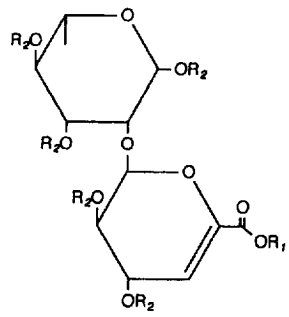
RESULTS AND DISCUSSION

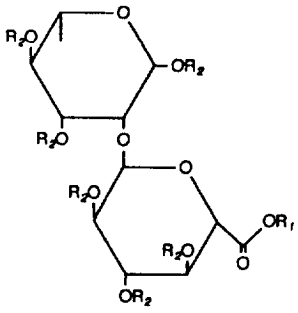
The structure–activity relationship of lepidimoide, its analogues and some sugars shown in Fig. 1 was studied by means of the *A. caudatus* hypocotyl elongation test (Table 1). The most active compound was compound 2 [2-*O*-(α-D-glucopyranosyl)-L-rhamnose], which is composed of rhamnose and glucose. It caused about 200% or greater promotion in the *A. caudatus* hypocotyl elongation at 3×10^{-3} M. However, when the R_2 groups of the disaccharide were changed to benzyl groups and the R_1 group changed to an acetyl group, the activity was completely lost. The carboxylic acid free type (**13**) of lepidimoide (Na type, **14**) showed as high growth-promoting activity as lepidimoide did. The methyl ester (**12**) of lepidimoide exhibited no activity. However, the acetylation of compound **12** revealed activity. On the other hand, the hydroxylated compound (**6**) of lepidimoide showed a low activity (*ca* 10%) compared with lepidimoide. Compound **6** and the free carboxylic acid type (**5**) showed low activities, and the methylated compound



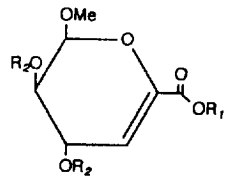
	R_1	R_2
1	Ac	Bn
2	H	H



	R_1	R_2
11	Me	Ac
12	Me	H
13	H	H
14	Na	H

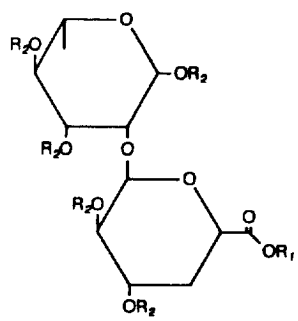


	R_1	R_2
3	Me	Ac
4	Me	H
5	H	H
6	Na	H



	R_1	R_2
15	Me	Ac
16	Me	H
17	H	H
18	Na	H

19 α-D-Galacturonic acid
20 L-(+)-Rhamnose
21 D-(+)-Glucose
22 Saccharose



	R_1	R_2
7	Me	Ac
8	Me	H
9	H	H
10	Na	H

Bn = benzyl group
Ac = acetyl group
Me = methyl group

Fig. 1. Structures of lepidimoide and its analogues applied to *Amaranthus caudatus* L.

(**4**) decreased the activity, but the decreased activity was not recovered by acetylation. The deoxy-types (**7**–**10**) of compounds **3**–**6** showed higher activity than that of the latter compounds, except that the methylated compound showed no activity, although the deoxy-types showed lower activity than that of lepidimoide. The activities of monosaccharides were also studied. 1-Methoxy-4-deoxyuronic acid (**17**) and its Na type (**18**) showed about 30% lower activity than that of lepidimoide. The methylated type (compound **16**) and the acetylated type (**15**) of compound **17** had little activity. In addition, the activities of α-D-galacturonic acid and L-(+)-rhamnose, which are component sugars of lepidimoide, were studied. α-D-Galacturonic acid (**19**), L-(+)-rhamnose (**20**) and their mixture exhibited weak or no activity, respectively. D-Glucose and L-(+)-rhamnose, which are component sugars of a potent high active compound (**2**), were also studied. These sugars and their mixture also had low activity or were inactive except that D-glucose showed a considerable activity at 3×10^{-3} M.

These data suggest that the structural requirements of lepidimoide for plant growth-promoting activities obtained by the *A. caudatus* hypocotyl test are the uronic acid derivatives on the α,β-unsaturated carboxylate bonded to rhamnose via an α-glucoside linkage and a double bond in the C-4,5 position in uronic acid.

Table 1. Promoting activities of lepidimoide, its analogues and some sugars on the growth of *Amaranthus caudatus* L. hypocotyls

Compounds	Activity (% of control)			
	3×10^{-3} M	10^{-3} M	3×10^{-4} M	10^{-4} M
1	108 ± 1.1	101 ± 2.6	103 ± 7.0	111 ± 1.8
2	204 ± 13.1	167 ± 7.1	148 ± 8.0	117 ± 3.9
3	88 ± 7.1	93 ± 10.1	110 ± 1.1	108 ± 3.5
4	110 ± 7.1	112 ± 1.9	113 ± 1.2	102 ± 6.4
5	125 ± 4.6	114 ± 0.7	111 ± 8.2	107 ± 3.2
6	128 ± 2.5	122 ± 4.1	113 ± 2.4	105 ± 7.8
7	149 ± 12.8	123 ± 2.9	117 ± 6.6	105 ± 7.8
8	114 ± 3.5	105 ± 2.4	102 ± 3.7	107 ± 0.4
9	145 ± 8.1	129 ± 2.0	114 ± 4.8	114 ± 2.8
10	143 ± 2.8	119 ± 7.2	106 ± 5.0	99 ± 5.3
11	161 ± 6.7	155 ± 14.2	136 ± 11.6	100 ± 1.8
12	107 ± 2.1	114 ± 4.3	109 ± 4.8	109 ± 0.4
13	151 ± 4.9	138 ± 2.5	122 ± 6.1	105 ± 2.1
14	156 ± 9.2	137 ± 4.0	120 ± 5.2	105 ± 1.8
15	124 ± 3.2	105 ± 1.7	101 ± 5.3	103 ± 5.7
16	121 ± 2.5	110 ± 12.8	105 ± 6.9	102 ± 6.7
17	136 ± 4.2	141 ± 7.5	117 ± 0.5	97 ± 3.5
18	139 ± 1.4	126 ± 2.7	111 ± 2.6	105 ± 3.2
19	118 ± 1.1	112 ± 1.8	96 ± 0.7	
20	94 ± 8.8	98 ± 0.4	99 ± 4.6	
21	139 ± 3.9	117 ± 1.8	109 ± 1.8	
22	110 ± 0.7	110 ± 2.1	98 ± 0.4	
19 + 20	104 ± 4.2	89 ± 9.5	86 ± 9.2	
20 + 21	127 ± 0.6	124 ± 5.3	113 ± 6.6	

Data represent mean ± SE.

EXPERIMENTAL

Materials. Lepidimoide and its analogues were synthesized according to the procedure reported in ref. [4].

Bioassay. The range of concn of the test soln was from 3×10^{-3} to 10^{-4} M. A soln of 0.4 ml of the test compound in H_2O was directly absorbed on a filter paper (Toyo filter No. 1) in a Petri dish (27 mm). The compounds, which are difficult to solubilize in H_2O, were dissolved in Me_2CO and absorbed on the filter paper in the dishes. After evapn at room temp., 0.4 ml H_2O was added. 8 seeds of *A. caudatus* were spread on the filter paper in a Petri dish, and cultured in the dark at 25° for 4 days, and then hypocotyl length was measured.

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